

REPRODUCTIVE FITNESS AND POLLINATION BIOLOGY
OF THE FOUR FLORAL COLOR MORPHS OF
CLARKIA AMOENA VAR. *PACIFICA*

ANDREA C. FOUST AND JENNIFER J. BUTLER¹
Willamette University, Salem, OR 97301

ABSTRACT

We studied four floral color morphs of the annual *Clarkia amoena* var. *pacifica* to determine whether variation in reproductive fitness and pollination biology accompanied floral color variation. Reproductive variables examined included insect visitation, pollen production and deposition and seed set. Pollinator visitation rates were not significantly different among color morphs in observation plots located in the two study areas. However, the white morph, which occurred in a separate population, differed from the other morphs in that it received visits from a greater variety of insects. The white morph also produced 3.3 times more pollen than the other three morphs and pollen deposition on the stigmas of the white morph was 7.2 times greater than in the other three morphs. In addition, while most within morph comparisons of the number of seeds produced by open-pollinated, self-pollinated and cross-pollinated flowers were insignificant, seed set for the white morph was significantly less in outcrossed flowers. Meanwhile, among morphs, the white flowers produced more seed per fruit ($\bar{x} = 101.1$, $SD = 16.3$) than the purple-spotted ($\bar{x} = 68.2$, $SD = 33.8$) or white-spotted ($\bar{x} = 64.2$, $SD = 27.4$) color morphs under self-pollination. This study suggests potential links between pollinator diversity, seed set, and population differentiation in a plant that appears to combine autogamous and xenogamous elements in its breeding system.

Floral color variation within and among plant populations is often related to and accompanied by other types of variation (Miller 1981; Waser and Price 1981; Galen 1989; Bosch 1992). In addition, when pollinators discriminate among floral variants, pollinator-mediated selection may play a role in the evolution of further floral variation (Miller 1981; Galen 1989; Robertson and Wyatt 1990). Discrimination among morphs by pollinators sometimes influences male reproductive characteristics, such as the number of pollen grains produced and the ability to deposit pollen on stigmas (Bosch 1992; Stanton et al. 1986). Differential pollen deposition on stigmas can also lead to variation in female fitness, measured as seed production (Waser and Price 1981; Galen 1989). Furthermore, if the spatial distribution of floral morphs is closely correlated with that of effective pollinators (Miller 1981; Galen 1989; Robertson and Wyatt 1990), pollinator-mediated selection may affect the development of distinct races (Miller 1981).

¹ Present address: University of Connecticut, Department of Ecology and Evolutionary Biology, Storrs, CT 06269.

Previous studies of the genus *Clarkia* have focused mainly on genetic variation (e.g., Soltis and Bloom 1991), evolutionary mechanisms (e.g., Lewis 1953) and pollinators of *Clarkia* (MacSwain et al 1973). Less attention has been given to the variation in reproductive fitness among floral color morphs and how this relates to pollination biology (Jones 1994). The purpose of this study was to examine whether floral color variation in *Clarkia amoena* var. *pacifica* was accompanied by variation in reproductive fitness and the ability to attract pollinators. Specific questions we addressed were: 1) Do differences in pollinator visitation rates occur among the four color morphs? 2) Are the different color morphs visited by the same insects? 3) Is variation in female reproductive fitness, pollen production and pollen deposition present?

METHODS

Clarkia amoena var. *pacifica* is an annual that occurs as four floral color morphs: white, purple, purple-spotted and white-spotted (spotted individuals have a magenta spot centrally located on each white or purple petal). We were able to locate two sites on the Oregon coast that contained *C. amoena* var. *pacifica*, Cascade Head and Cape Lookout. Cascade Head, a grassy promontory located 7 miles north of Lincoln City, Oregon, represents the only known site containing all four color morphs. We studied two populations of *C. amoena* var. *pacifica* which occur separately at Cascade Head. One site, located inland approximately 0.8 km, contains only the white morph. The second site is located along the face of the headland and contains the white-spotted, purple-spotted and purple morphs.

Pollinator study. We estimated pollinator visitation rates using 44, one meter square observation plots that were randomly located across both sites during the 1993 flowering season (May–August). Plots were observed using 20 minute time intervals. We observed plots throughout the day for all sites and color morphs, in both sunny and cloudy weather ($n = 8$ days of observation). Flowers were diurnal and therefore nocturnal pollinators were not considered. We observed 18 plots that contained only the white morph while we observed 26 plots that contained a mixture of the other three morphs. Four people assisted in pollinator studies which allowed some simultaneous observation of different plots; observers were also rotated among sites A and B. An insect was considered to be a potential pollinator if it made direct contact with the anthers. For each visit, we recorded the type of insect, the color morph visited, the number of flowers sampled, and the length of the visit.

Because we were interested in visitation rates in natural populations and since plots were randomly located in both sites, plant densities varied among plots, ranging from 3 to 57 *Clarkia* per plot.

Variability in color morph frequency among plots was monitored by recording the number of flowers of each morph per plot prior to each observation period. To determine whether flowering density affected pollinator visitation frequency we tested for a possible correlation between flower number and pollinator frequency. In addition, we compared differences in the average percentage of flowers of each morph visited per plot during the 20 minute observation periods using Chi-square.

Pollen production and deposition. To examine pollen production in the four floral color morphs we collected one flower from each of 20 randomly selected individuals of each color morph just prior to pollen release. Flowers were placed individually in glycine bags and stored temporarily in a lab desiccator. For pollen counts, we soaked individual flowers in water and placed these on a 350 um plankton sieve. We then washed pollen from each flower with a forceful stream of water. When microscopic examination of the sieve and anthers revealed that the pollen from all the anthers had been washed into the beaker below, the volume of water was recorded. Using a micropipetor we removed 20 (2 ml) samples from each beaker and counted the number of pollen grains using a dissecting microscope. We added glycerine drops prior to counts and pollen was stirred magnetically to ensure an even distribution and to prevent pollen grains from adhering to one another. (This was especially important because SEM photos [Phillips xl-20] revealed pollen grains to be connected by viscin threads.) The average number of pollen grains/ml was calculated and then multiplied by the total volume of water in the beaker to find the total number of pollen grains produced by each flower.

To determine the amount of pollen deposited on stigmas we sampled 10 senescent flowers (all from distinct individuals) of each color morph. Senescent flowers were defined as those that remained closed all day and appeared wilted. We removed the stigmas from these flowers and stored them individually at -85°C . For pollen counting, each stigma was placed in a depression slide with 10% hydrochloric acid and heated for about three minutes on a hot plate. Stigmas were then easily crushed between two slides, which were rubbed together to distribute the pollen across the slide. A grid placed on top of the slide aided microscope counts of the number of pollen grains.

Breeding system study.

Open-pollinated individuals.—to assess seed set under field conditions that allowed for insect pollination and autofertility, we bagged 10 green capsules from different, randomly selected plants of each color morph prior to seed release. After maturity, we re-

corded the number of filled and unfilled seed. Unfilled and filled seeds were clearly different.

Self-pollinated individuals.—In a previous study Lewis and Lewis (1955) found *C. amoena* var. *huntiana* collected in the field and grown under greenhouse conditions was self-pollinating. Thus, to determine the extent of autofertility in the absence of insects, we bagged non-emasculated flowers of each color morph with a fine nylon mesh netting prior to pollen release and stigma receptivity. Once the flowers had senesced, we removed nets and later collected the mature capsules. We recorded the number of filled and unfilled seed produced in each capsule for 10 capsules of each color morph.

Outcrossed individuals.—We evaluated the seed set attributed to allogamy by emasculating 10 flowers on randomly selected plants of each color variant prior to stigma receptivity. Pollen left on the petals was removed by using a brush to insure that the flower would not be self-pollinated. Once flowers had senesced, we marked and bagged 10 capsules of each morph for counts of filled and unfilled seed. On average, three flowers of any given plant were simultaneously releasing pollen.

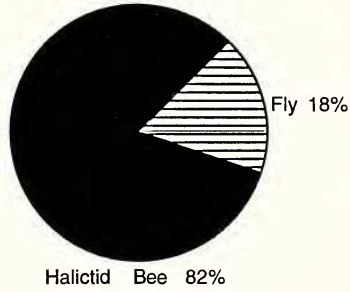
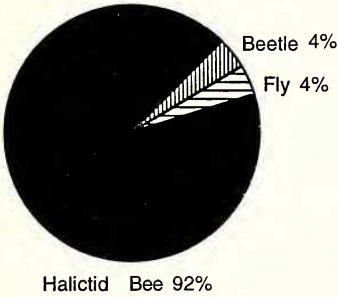
We examined variation in means among the color morphs using a two-way analysis of variance with color morph, pollination treatment and their interaction as the main effects. When variation was found to be present we applied the Tukey test (Zar 1984) to determine significant differences between means.

RESULTS

Pollinator visitation. The 44 observation plots contained an average of 18.8 *C. amoena* var. *pacifica* individuals. We compared the frequency of observed visits for different morphs by dividing the number of visits to each morph during the 20 minute observation interval by the number of flowers of that color morph in a given plot; we then averaged these results for all plots. Of the total number of purple-spotted flowers observed, on average 14.5% were visited during each 20 minute observation period while 9.6% of the white-spotted, 14.9% of the purple and 7.5% of the white flowers observed were visited. Visitation rates were not significantly different among the color morphs ($\chi^2 = 3.44$, $df = 3$, $P > 0.05$). However, the types of potential pollinators present on color morphs varied. Purple-spotted, purple and white-spotted flowers received 82–92% of their visits from halictid bees, whereas white flowers received visits from a greater variety of insects, including bumblebees and syrphid flies (Fig. 1). We observed pollinators visiting flowers of multiple colors on one foraging bout, however, we did not observe pollinators visiting more than one flower on an individual plant during one foraging bout. Although there was a significant difference between

PURPLE-SPOTTED

WHITE-SPOTTED



PURPLE

WHITE

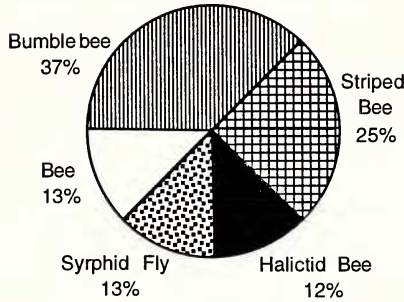
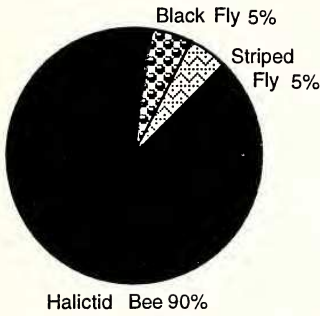


FIG. 1. Proportion of visits attributed to each of the potential pollinators for the four color morphs of *Clarkia amoena* var. *pacifica*.

plant densities in the mixed color morph plots versus the white color morph plots ($\chi^2 = 4.19$, $df = 1$, $P < 0.05$), there were no significant correlations between plant density and pollinator visitation rates ($r = 0.039$, $P = 0.85$ for the mixed plots; $r = 0.059$, $P = 0.82$ for the white plots).

Pollen production and deposition. Significant variation was present in pollen production (ANOVA, $F = 41.39$, $df = 7$, $P < 0.05$) and in the number of pollen grains deposited on stigmas among the color morphs (ANOVA, $F = 26.17$, $df = 36$, $P < 0.05$). Pollen production in the white morph ($\bar{x} = 5617$, $SD = 2118$ grains per flower) was, on average, 3.3 times greater than in the other three morphs (purple-spotted: $\bar{x} = 1574$, $SD = 859$; purple: $\bar{x} = 2134$, $SD = 1060$; white-spotted: $\bar{x} = 1415$, $SD = 725$) which were not significantly different from one another. White flowers also received significantly more pollen ($\bar{x} = 1942$, $SD = 996$ grains per stigma)

TABLE 1. MEAN NUMBER AND STANDARD DEVIATION OF SEED SET PER FRUIT AMONG OPEN-POLLINATED, SELF-POLLINATED AND OUTCROSSED INDIVIDUALS OF EACH COLOR MORPH OF *CLARKIA AMOENA* VAR. *PACIFICA*.

	Open-pollinated	Self-pollinated	Outcrossed
Purple-spotted	72.5 ± 26.2	68.2 ± 33.8	75.5 ± 17.1
Purple	69.5 ± 39.7	89.7 ± 22.7	60.0 ± 40.7
White-spotted	51.6 ± 25.0	64.2 ± 27.4	61.3 ± 21.2
White	89.8 ± 28.0	101.1 ± 16.3	59.3 ± 32.6

than the other three morphs (purple-spotted: \bar{x} = 292, SD = 190; purple: \bar{x} = 259, SD = 151; white-spotted: \bar{x} = 251, SD = 148).

Breeding system study. Pollination treatment and flower color both had a significant effect on seed production. However, there was no significant effect due to the interaction of flower color and pollination treatment. (ANOVA, F = 11.4, df = 3 for flower color, F = 3.3, df = 2 for pollination treatment, P < 0.05. For the interaction of the flower color and pollination treatment F = 1.31, df = 6, P = 0.26)

Within morph comparisons.—The only significant within color morph variation in seed production among the pollination treatments occurred in the white morph. The white morph produced significantly less seed per fruit under the outcrossing treatment (ANOVA, F = 6.64, df = 27, P < 0.05; Tukey P < 0.05; Table 1).

Between morph comparisons.—Between morphs, the only significant difference occurred under the self-pollination condition. The white morph produced significantly more seed per fruit under this treatment than both the purple-spotted and white-spotted morphs (ANOVA, F = 4.62, P < 0.05; Tukey at P < 0.05; Table 1).

DISCUSSION

Variation in seed set, pollen production and deposition, and pollinator diversity does accompany differences in floral color in *C. amoena* var. *pacifica*. Specifically, the white morph differed from the other morphs in the types of pollinators present, pollen production and deposition, and some aspects of seed set. However, visitation rates by insects did not significantly differ among the morphs, and habitat differences may account for some of the differences observed.

Pollinator visitation. Even though insect visitation rates did not differ among color morphs, a greater diversity of pollinators visited the white morph. This may have resulted from several factors. First, certain insects preferentially visit particular floral colors (Pederson 1967; Heinrich 1974; Mogford 1974). For example, Pederson (1967)

found that solitary bees visiting alfalfa selectively avoided white flowers, whereas Mogford (1974) found that bumblebees preferred white flowers of *Cirsium palustre*. In this study, visits by halictids, a type of solitary bee, represented only 12% of the visits to the white morph yet constituted between 82–92% of the visits for the other three morphs. In contrast, bumblebees were observed only on the white morph. Thus, color preferences of pollinators may play a role in visitation patterns to *C. amoena* var. *pacifica*.

Secondly, the wider array of visitors observed on the white flowered morph may be related to its greater production of pollen, an important primary attractant for many pollinators. However, whereas pollen production may have influenced the diversity of insects observed on various color morphs, the overall visitation rates were not different among morphs. Additional studies are needed to more clearly differentiate the potential effects of pollen production on both pollinator visitation rates and pollinator diversity.

Additionally, high pollen production and larger flower size often covary; in *Raphanus* and *Polemonium* the combination of these two factors may enhance the attractiveness of plants to pollinators, especially bumblebees (Stanton and Preston 1988; Galen and Newport 1987). Similarly, in *C. amoena* var. *pacifica*, the flowers of the white that produce more pollen are also significantly larger than those of the other three morphs (Butler and Foust, unpublished observation).

Finally, variation in habitat may explain some of the differences in pollinator visitation patterns. Differences among pollination systems in some species of *Clarkia* appear to be mainly ecological; where the plants are restricted to different habitats, the pollinators may differ (MacSwain et al, 1973). At Cascade Head, plants of the white morph occur among tall grasses and forbs (approximately 1m tall) while the vegetation in the mixed population is largely dominated by red fescue and a diverse array of forbs (approximately 0.3 m tall). Casual observations of pollinators from previous years (Kephart, personal communication) also suggest a greater diversity of pollinators exists in the ungrazed headland. However, if habitat rather than floral factors were responsible for the observed differences in pollinator diversity, one would predict that the biotically rich area where the mixed population occurs (consisting of ungrazed coastal prairie) would have yielded the highest pollinator diversity. Instead, the white morph in the less diverse site supported a more varied pollinator fauna. Further experimental studies are needed to distinguish among pollinator, floral, and habitat based variables.

A possible outcome of differential visitation to varied populations of a species is reproductive isolation and/or evolutionary divergence (Miller 1981; Grant 1949; Stebbins 1971). Some evidence presented here supports possible reproductive isolation for the white morph and a likelihood of considerable interbreeding among the co-occur-

ring color morphs. Observations of insect flights showed that pollen transfer readily occurs between the purple-spotted, purple, and white-spotted morphs. Similarly, Jones (1994) found that pollinators moved randomly between plants of spotted and unspotted morphs of *Clarkia gracilis*. In contrast, while pollen transfer between the white morph and the other three morphs of *C. amoena* var. *pacifica* is possible, it is likely reduced by the distance separating these populations (but see Kirkpatrick and Wilson 1988; Broyles and Wyatt 1990). In our study of *C. amoena* var. *pacifica*, reproductive isolation would be facilitated by spatial distance, differential pollinator visitation, evidence of possible autogamy in the white morph, and by the genetic differentiation for the white morph implied by electrophoretic studies (Butler and Foust 1996). These hypotheses merit further testing by combining techniques such as paternity analysis with studies of the foraging patterns of marked insects.

Pollen production and deposition. In the white morph, pollen production and deposition were higher than in the other three morphs. Theoretically, the ability to produce more pollen serves to attract more pollinators, thereby potentially increasing the number of grains deposited on stigmas (Stanton and Preston 1988). In this study, however, greater pollen production in the white morph did not result in greater insect visitation, although it was associated with greater pollinator diversity and the presence of bumblebees. Thus, the greater number of pollen grains deposited on stigmas of the white morph may instead reflect the high efficiency of bumblebees with respect to pollen transfer (Waser 1982).

Another consequence of the greater pollen production in the white morph may be enhanced self-pollination leading to higher seed set. One could argue that the significance of higher pollen deposition for reproductive success is probably somewhat limited, since stigmas of all color morphs generally received more pollen grains than the number of seed produced. However, some studies have found that increased pollen deposition may lead to increased progeny vigor (Winsor et al. 1987; Schlichting et al. 1990). Further studies on germination rates, seedling growth rate and other similar characters would give important information regarding the possible consequences of the increased pollen loads of the white morph.

Female fitness. Significant variation in female fitness within floral color variants was limited to greater seed set by the white morph in open and autogamously pollinated flowers. Because autogamy was possible only under these two conditions, the high pollen production in the white morph may influence seed set primarily via enhanced self pollination. While some inbreeding depression is likely with such a pollination system, it does not appear to compromise adequate seed set in *C. amoena* var. *pacifica*. Also, in adverse weather

(e.g. rain and high winds common at Cascade Head), a pollination system that is not pollinator-limited may be especially important (Oleson and Warncke 1992). In *C. amoena* var. *pacifica*, the equivalent heights of the anthers and stigmas and incomplete protandry also facilitate self-pollination. We observed contact of dehiscing anthers and receptive stigmas under varying weather conditions, but most commonly during rainy periods when few pollinators were present.

The increased seed set under the self-pollination condition for the white morph also highlights the potential importance of self-pollination in clarkias with mixed-mating systems. In *Clarkia gracilis* ssp. *sonomensis* self pollen deposition is common, but stigmas exposed to pure self and outcross pollen produce similar number of seeds while mixtures of self and outcross pollen produce three times more outcrossed as selfed offspring (Jones 1994). Thus, the success of self and outcross pollen in producing seed may be dependent on the presence or absence of competition, which can be influenced by the number and type of pollen grains on each stigma in *C. amoena* var. *pacifica*.

The importance of self-pollination within the white morph merits further study based on differential seed set. In other color morphs, however, filled seed set under the three different pollination conditions did not vary. The breeding system of *Clarkia amoena* var. *pacifica* overall combines both autogamy and xenogamy.

ACKNOWLEDGMENTS

We thank S. Kephart and J. Koprowski for their encouragement and advice throughout the study. S. Kephart, J. Koprowski, K. Holsinger and two anonymous reviewers also contributed helpful comments on the manuscript. We also thank H. Jones and A. Urton for field assistance, S. Hawke for SEM photos of pollen, and N. Perigo for logistical support. Funding was provided through a Willamette University Undergraduate Research Grant and the Hardman Foundation. We thank The Nature Conservancy for access to Cascade Head Preserve and the Forest Service for housing near the study area. This study represents part of a senior thesis submitted to Willamette University by Andrea Foust.

LITERATURE CITED

- BOSCH, J. 1992. Floral biology and pollinators of three co-occurring *Cistus* species (Cistaceae). *Botanical Journal of the Linnean Society* 109:39-55.
- BROYLES, S. B. and R. WYATT. 1990. Paternity analysis in a natural population of *Asclepias exaltata*: multiple paternity, functional gender, and the "pollen donation hypothesis." *Evolution* 44:1454-1468.
- BUTLER, J. J. and A. C. FOUST. 1996. Morphological and electrophoretic variation among the four floral color morphs of *Clarkia amoena* var. *pacifica* (Onagraceae). *Madrono* 43:235-244.
- GALEN, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* 43: 882-890.
- and M. E. A. NEWPORT. 1987. Pollination in floral scent morph of *Polemo-*

- nium viscosum*: a mechanism for disruptive selection of flower size. *Evolution* 41:599–606.
- GRANT, V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* 3:82–97.
- HEINRICH, B. 1974. Bee flowers: a hypothesis on flower variety and blooming times. *Evolution* 29:325–334.
- JONES, K. N. 1994. Nonrandom mating in *Clarkia gracilis* (Onagraceae): a case of cryptic self-incompatibility. *American Journal of Botany* 81:195–198.
- KIRKPATRICK, K. J. and H. D. WILSON. 1988. Interspecific gene flow in *Cucurbita*: *C. texana* vs. *C. pepo*. *American Journal of Botany* 75:519–527.
- LEWIS, H. 1953. The mechanism of evolution in the genus *Clarkia*. *Evolution* 7:1–20.
- and M. E. LEWIS. 1955. The genus *Clarkia*. University of California Publications in Botany 20:693–712.
- MACSWAIN, J. W., P. H. RAVEN, and R. W. THORP. 1973. The comparative behavior of bees on Onagraceae IV. *Clarkia* bees of the Western United States. University of California Publications in Entomology 70:1–80.
- MILLER, R. B. 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* 35:763–774.
- MOGFORD, D. J. 1974. Flower colour polymorphism in *Cirsium palustre*. I. *Heredity* 33:241–256.
- OLESON, L. and E. WARNCKE. 1992. Breeding system and seasonal variation in seed set in a population of *Potentilla palustris*. *Nordic Journal of Botany* 13:378–380.
- PEDERSON, M. W. 1967. Cross-pollination studies involving three purple-flowered alfalfas, one white-flowered line, and two pollinator species. *Crop Science* 7:59–62.
- ROBERTSON, J. L. and R. WYATT. 1990. Evidence for pollination ecotypes in the yellow-fringed orchid, *Platanthera ciliaris*. *Evolution* 44:121–133.
- SCHLICHTING, C. D., A. G. STEPHENSON, and L. E. SMALL. 1990. Pollen loads and progeny vigor in *Cucurbita pepo*: the next generation. *Evolution* 44:1358–1372.
- SOLTIS, P. and W. BLOOM. 1991. Allozymic differentiation between *Clarkia nitens* and *C. speciosa* (Onagraceae). *Systematic Botany* 16:399–406.
- STANTON, M. L. and R. E. PRESTON. 1988. Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany* 75:528–539.
- , A. A. SNOW, and S. N. HANDEL. 1986. Floral evolution: attractiveness to pollinators increases male fitness. *Science* 232:1625–1627.
- STEBBINS, G. L. 1971. Adaptive radiation of reproductive characteristics in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics* 1:307–326.
- WASER, N. M. 1982. A comparison of distances flown by different visitors to flowers of the same species. *Oecologia* 55:251–257.
- and M. V. PRICE. 1981. Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* 35:376–390.
- WINSOR, J. A., L. E. DAVIS, and A. G. STEPHENSON. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. *American Naturalist* 129:643–656.
- ZAR, J. H. 1984. *Biostatistical Analysis*. Prentice Hall, NJ.

(Received 18 Aug 1994; revision accepted 20 June 1995)